इंटरनेट मानक

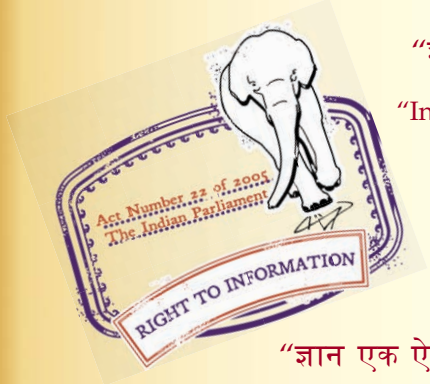
Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS 5960-13 (1988): Meat and meat products - Methods of test, Part 13: Determination of polyphosphates [FAD 18: Slaughter House and Meat Industry]



"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

BLANK PAGE

IS 5960 (Part 13) : 1988
ISO 5553 : 1980
(Reaffirmed 2000)

Indian Standard

MEAT AND MEAT PRODUCTS — METHODS OF TEST

PART 13 DETECTION OF POLYPHOSPHATES

UDC 637·5 : 543·822

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

January 1990

Price Group 3

Indian Standard

MEAT AND MEAT PRODUCTS — METHODS OF TEST

PART 13 DETECTION OF POLYPHOSPHATES

NATIONAL FOREWORD

This Indian Standard (Part 13), which is identical with ISO 5553 : 1980 'Meat and meat products — Detection of polyphosphates (reference method)', issued by the International Organization for Standardization (ISO), was adopted by the Bureau of Indian Standards on 25 November 1988 on the recommendation of the Meat Industry Sectional Committee (AFDC 18) and approval of the Agricultural and Food Products Division Council.

This Indian Standard has been issued in several parts, which are listed on page 2.

In the adopted standard, certain terminology and conventions are not identical with those used in Indian Standard; attention is especially drawn to the following:

a) Wherever the words 'International Standard' appear, referring to this standard, they should be read as 'Indian Standard'; and

b) Comma (,) has been used as decimal marker, while in Indian Standards, the current practice is to use point (.) as the decimal marker.

Though the adopted standard prescribes 'reference method', the same is, however, applicable as a routine method in this Indian Standard.

CROSS REFERENCE

In this Indian Standard, the following International Standard is referred to. Read in its place the following:

International Standard	*Corresponding Indian Standard*	*Degree of Correspondence*
ISO 3100 Meat and meat products — Sampling	IS : 1723-1973 Specification for pork (*first revision*)	Partly equivalent
	and	
	IS : 1743-1973 Specification for mutton and goat meat canned in brine (*first revision*)	Partly equivalent

IS 5960 Methods of test for meat and meat products:

IS 5960 (Part 1) : 1970 Determination of nitrogen content

IS 5960 (Part 2) : 1970 Determination of ash

IS 5960 (Part 3) : 1970 Determination of total fat content

IS 5960 (Part 4) : 1970 Determination of free fat content

IS 5960 (Part 5) : 1970 Determination of moisture content

IS 5960 (Part 6) : 1971 Determination of chloride content

IS 5960 (Part 7) : 1974 Determination of nitrite content

IS 5960 (Part 8) : 1974 Determination of nitrate content

IS 5960 Meat and meat products — Methods of test:

IS 5960 (Part 9) : 1988/ ISO 2294 : 1974 Determination of total phosphorus content

IS 5960 (Part 10) : 1988/ ISO 2917 : 1974 Measurement of *p*H

IS 5960 (Part 11) : 1988/ ISO 4133 : 1979 Determination of glucono-delta-lactone content

IS 5960 (Part 12) : 1988/ ISO 4134 : 1978 Determination of L—(+)- glutamic acid content

IS 5960 (Part 13) : 1988/ ISO 5553 : 1980 Detection of polyphosphates

IS 5960 (Part 14) : 1988/ ISO 5554 : 1978 Determination of starch content

IS 5960 (Part 15) : 1989/ ISO 3496 : 1978 Determination of L (—) hydroxyproline content

1 Scope

This International Standard specifies a method for the detection of linear condensed phosphates in meat and meat products by thin layer chromatographic separation.

2 Field of application

Since polyphosphates are gradually hydrolyzed by enzymes present in the meat or meat product and during heat treatment of the meat or meat product, this International Standard only applies to the detection of added polyphosphates that are still present in the sample at the time of investigation.

3 Reference

ISO 3100, *Meat and meat products — Sampling.*

4 Principle

Extraction of the meat or meat product with trichloroacetic acid. Clearing of the serum obtained with ethanol/diethyl ether mixture. Separation of the phosphates by thin layer chromatography and detection of polyphosphates by spraying with reagents for colour development.

5 Reagents

All reagents shall be of recognized analytical quality. Distilled water or water of at least equivalent purity shall be used.

Warning — All appropriate safety precautions shall be observed when carrying out the procedures specified in this International Standard.

5.1 Trichloroacetic acid.

5.2 Diethyl ether.

5.3 Ethanol, 95 % (*V*/*V*).

5.4 Cellulose powder, for thin layer chromatography.

5.5 Soluble starch.

5.6 Reference mixture.

Dissolve in 100 ml of water.

— 200 mg of sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$),

— 300 mg of tetrasodium diphosphate decahydrate ($Na_4P_2O_7.10H_2O$),

— 200 mg of pentasodium triphosphate ($Na_5P_3O_{10}$), and

— 200 mg of sodium hexametaphosphate $(NaPO_3)_x[x > 10]$.

The reference mixture is stable at 4 °C for at least 4 weeks.

5.7 Developing solvent.

Mix 140 ml of isopropyl alcohol, 40 ml of a 135 g/l solution of trichloroacetic acid, and 0,6 ml of ammonium hydroxide, ϱ_{20} = 0,90 g/ml; about 25 % (*m*/*m*) solution.

Keep the solvent in a tightly closed bottle.

5.8 Spray reagent I.

Mix equal volumes of a 75 g/l solution of ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}.4H_2O]$ and concentrated nitric acid (ϱ_{20} = 1,40 g/ml) and dissolve 10 g of tartaric acid in 100 ml of this mixture.

Prepare the reagent on the day of use.

5.9 Spray reagent II.

Dissolve 0,5 g of 1-amino-2-naphthol-4-sulphonic acid in a mixture of 195 ml of a 150 g/l solution of sodium disulphite (sodium metabisulphite; $Na_2S_2O_5$) and 5 ml of a 200 g/l solution of sodium sulphite (Na_2SO_3). Dissolve 40 g of sodium acetate trihydrate ($NaOOCCH_3.3H_2O$) in this mixture.

Store the reagent in a tightly closed brown bottle in the refrigerator. Discard the solution after 1 week.

6 Apparatus

Usual laboratory equipment not otherwise specified and the following items :

6.1 Glass plates, thoroughly degreased, 10 cm × 20 cm.

6.2 Spreading device, for preparing layers of 0,25 mm thickness. If such a device is not available, ready-to-use thin-layer plates with layer thicknesses of 0,25 mm can be used provided that starch is used as the binder. Plates containing gypsum (calcium sulphate) are not suitable.

6.3 Laboratory mixer.

6.4 Desiccator.

6.5 Mechanical meat mincer, laboratory size, fitted with a plate with holes of diameter not exceeding 4 mm.

6.6 Fluted filter paper, of diameter 15 cm.

6.7 Micro-pipette, 1 µl, or **micro-syringe** with micrometer screw and bent glass tip.

6.8 Paper-lined glass tank, of appropriate dimensions, with tightly fitting lid, for development of thin-layer chromatograms.

6.9 Hair-dryer, capable of providing either an air stream at room temperature or a warm air stream.

6.10 Sprayer.

6.11 Oven, capable of being controlled at 60 °C.

7 Sample

7.1 Proceed from a laboratory sample of at least 200 g. See ISO 3100.

7.2 Prepare the test sample on the day of its receipt in the laboratory.

8 Procedure

8.1 Preparation of thin-layer plates

Dissolve 0,3 g of starch (5.5) in 90 ml of boiling water. Cool, add 15 g of cellulose powder (5.4) and homogenize in the laboratory mixer (6.3) for 1 min.

Apply this slurry onto glass plates (6.1) with the spreading device (6.2) adjusted to obtain a layer of 0,25 mm.

Air-dry the plates undisturbed for 60 min at room temperature and heat them finally for 10 min at 100 °C.

Store the plates in the desiccator (6.4).

Alternatively, ready-to-use thin-layer plates may be used (see 6.2).

8.2 Preparation of the test sample

Homogenize the sample by passing it at least twice through the meat mincer (6.5) and by mixing. Keep it in a completely filled, air-tight, closed container and store it, if necessary, in a refrigerator. Analyse the sample as soon as possible, but in any case within 5 h.

8.3 Preparation of serum

8.3.1 Macerate 50 g of the test sample (8.2) with 15 ml of water at 40 to 60 °C in a beaker by means of a spatula or a flattened stirring rod until a homogeneous mass is obtained, but taking no more than 5 min.

8.3.2 Add 10 g of the trichloroacetic acid (5.1) and again mix thoroughly.

8.3.3 Immediately place in a refrigerator for 1 h and then collect the separated serum by decanting through the fluted filter paper (6.6).

8.3.4 If the filtrate is turbid, shake once with an equal volume of the diethyl ether (5.2). Remove the ether layer with a small pipette and add an equal volume of the ethanol (5.3) to the aqueous phase. Shake for 1 min. Allow the mixture to stand for a few minutes and filter through a fluted filter paper (6.6).

8.4 Chromatographic separation

8.4.1 Pour developing solvent (5.7) into the developing tank (6.8) to a depth of 5 to 10 mm and close the tank with its lid. Allow to stand for at least 30 min at ambient temperature, protected from sunlight and draughts.

8.4.2 Apply 3 µl of the serum, or 6 µl if the clearing procedure of 8.3.4 was carried out, to the cellulose layer (8.1) on a pencil line drawn at about 2 cm from the bottom. Keep the spots small by applying 1 µl at a time.

Use a warm air stream from the hair-dryer (6.9) for drying.

NOTE — Hot air should be avoided because of the danger of hydrolysis of phosphates.

8.4.3 In the same way, apply 3 µl of the reference mixture (5.6) to the plate at a distance of 1 to 1,5 cm from the sample spot, but at exactly the same distance from the bottom.

8.4.4 Remove the lid from the tank and quickly but carefully place the cellulose plate in the tank. Replace the lid immediately. Develop the plate at ambient temperature, protected from sunlight and draughts.

8.4.5 Continue the development until the solvent front has ascended approximately 10 cm from the pencil line. Remove the plate from the tank and dry for 10 min in the oven (6.11) controlled at 60 °C, or alternatively, for 30 min at ambient temperature, or in a stream of cold air.

8.5 Detection of phosphates

8.5.1 Place the plate vertically under a fume hood and spray the plate lightly but uniformly with spray reagent I (5.8).

Yellow spots appear immediately.

8.5.2 Dry the plate in a stream of warm air from the hair dryer (6.9). Subsequently heat in an oven for at least 1 h at 100 °C to remove the last traces of nitric acid. Remove the plate from the oven and verify the absence of the pungent smell of nitric acid.

8.5.3 Allow the plate to cool to room temperature and then replace it under the fume hood. Spray the plate lightly but uniformly with spray reagent II (5.9).

Blue spots appear immediately.

NOTE — Spraying with reagent II is not an absolute necessity. However, the intense blue spots produced by this reagent improve the detection considerably.

9 Interpretation

Compare the migration distances of the phosphate spots from the sample with those of the phosphates from the reference mixture.

An orthophosphate spot is always present. If the sample contained condensed phosphates, a diphosphate spot and/or spots of more highly polymerized phosphates are visible.

The R_F values of the phosphates in the reference mixture are :

orthophosphate	from 0,80 to 0,90
diphosphate (pyrophosphate)	from 0,50 to 0,60
triphosphate	from 0,25 to 0,35
hexametapolyphosphate (Graham's salt)	0,0

Generally the R_F values of the polyphosphates in extracts of meat and meat products are somewhat lower.

NOTE — Corrections for the differences in R_F values of the phosphates in the sample extract and in the reference mixture can be obtained by placing an extract of the fresh meat sample on the same plate. As fresh meat only contains monophosphates, the percentage correction can be obtained by comparison of the migration distances of this standard spot with the corresponding spot from the reference mixture.

10 Test report

The test report shall show the method used and the results obtained. It shall also mention all operating conditions not specified in this International Standard or regarded as optional, as well as any circumstances that may have influenced the results.

The report shall include all details necessary for complete identification of the sample.

PRINTED AT SIMCO PRINTING PRESS, DELHI (INDIA)